

Moths are not silent, but whisper ultrasonic courtship songs

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SUMMARY

Ultrasonic hearing is widespread among moths, but very few moth species have been reported to produce ultrasounds for sexual communication. In those that do, the signals are intense and thus well matched for long distance communication. By contrast, males of the Asian corn borer moth (Crambidae) were recently shown to whisper extremely low-intensity ultrasonic courtship songs close to females. Since low sound levels will prevent eavesdropping by predators, parasites and conspecific rivals, we predicted low intensity ultrasound communication to be widespread among moths. Here we tested 13 species of moths including members of the Noctuidae, Arctiidae, Geometridae and Crambidae. Males of nine species, 70%, produced broadband ultrasound close to females. Peak frequencies ranged from 38 to above 100 kHz. All sounds were of low intensity, 43–76 dB SPL at 1 cm [64±10 dB peSPL (mean ± s.d.), *N*=9 species]. These quiet and/or hyper-frequency ultrasounds are audible to nearby mates, but inaudible to unintended receivers. Although largely unknown because it is so inconspicuous, acoustic communication using low intensity ultrasound appears to be widespread among hearing moths. Thus, acoustic communication may be the norm rather than the exception.

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Key words: acoustic communication, courtship behavior, low sound level, moths, ultrasound.

INTRODUCTION

Most moths have tympanal ears sensitive to ultrasound. The tuning of moth hearing to bat calls (Fullard, 1998) as well as the deterioration of moth hearing in bat-free areas (Surlykke et al., 1998) indicates that ears probably evolved in response to selective pressures imposed by foraging bats (Hoy and Robert, 1996; Miller and Surlykke, 2001; Waters, 2003). The location and morphology of moth ears vary across moth superfamilies, indicating an independent development in each group after the divergence of moth superfamilies (Hoy and Robert, 1996; Greenfield, 2002; Yack, 2004).

Subsequent to the development of ears, a relatively small number of moth species developed sound producing organs, and used them either for defense against bats or rival males (Spangler, 1988; Conner, 1999; Greenfield, 2002; Waters, 2003). For instance, both sexes of tiger moths (Arctiidae) emit jamming or warning ultrasonic clicks in response to echolocation calls of bats (Surlykke and Miller, 1985; Barber and Conner, 2007; Ratcliffe and Nydam, 2008). In the lesser wax moth, *Achroia grisella* (Pylalidae), males produce loud ultrasonic clicks to attract female mates (Spangler, 1988). The ultrasounds used by these species are characterized by high sound pressure levels, with the peak equivalent sound pressure level (peSPL) at a distance of 1 cm ranging from 76 to 125 dB.

Production of ‘courtship’ sounds, the sounds produced after pair formation and before mating, has only rarely been reported in moths, e.g. in some arctiids (Conner, 1987; Krasnoff and Yager, 1988; Sanderford and Conner, 1990; Simmons and Conner, 1996; Sanderford et al., 1998) and pyralids (Spangler, 1985; Trematerra and Pavan, 1995). The acoustic communication between sexes has

not been verified in most cases, but one effect of the male songs seems to be to prompt their mates to accept mating, in some cases in conjunction with chemical signals as in the pyralid, *Galleria mellonella* (Spangler, 1985). Given that many arctiid moths produce ultrasonic clicks to counteract bats (Miller and Surlykke, 2001), sexual communication via ultrasounds is likely to be a secondary use of the ability to produce ultrasound (Endler and Basolo, 1998).

We recently added the Asian corn borer moth, *Ostrinia furnacalis* (Crambidae), to the list of moths that produce courtship songs. *O. furnacalis* shows mating behavior typical of the majority of moths. First, males of *O. furnacalis* are attracted toward a sex pheromone-releasing female. After landing near the female, the male approaches her with his wings fanning (<2 cm) and subsequently attempts to mate by bending his abdominal tip toward hers. Male moths were found to produce ultrasonic courtship songs of extremely low intensity (46 dB peSPL at 1 cm) during these copulation attempts (Nakano et al., 2006; Nakano et al., 2008). Since the use of quiet sound would be advantageous in avoiding eavesdropping by predacious bats and rival males, this result prompted us to predict that whispering of ultrasonic courtship songs is widespread among moths (Nakano et al., 2008; Nakano et al., 2009). In the present study, we tested our prediction by examining 13 tympanate moth species, belonging to diverse taxonomic groups, for the production of quiet ultrasonic courtship songs.

MATERIALS AND METHODS

Insects

We investigated the emission of courtship ultrasound in 13 tympanate moth species belonging to four families (three

superfamilies): five species of Noctuidae (Noctuoidea), the cotton bollworm *Helicoverpa armigera* Hübner, the cabbage armyworm *Mamestra brassicae* Linnaeus, the common cutworm *Spodoptera litura* Fabricius, *Herminia tarsicrinalis* Knoch and *Diomea cremata* Butler; three species of Arctiidae (Noctuoidea), the tiger moth *Spilosoma punctarium* Stoll, the fall webworm *Hyphantria cunea* Drury and *Eilema japonica* Leech; one species of Geometridae (Geometroidea), the Japanese giant looper *Ascotis selenaria cretacea* Butler; and four species of Crambidae (Pyraloidea), the rice stem borer *Chilo suppressalis* Walker, the mulberry pyralid *Glyphodes pyloalis* Walker, *Palpita nigropunctalis* Bremer and *Spoladea recurvalis* Fabricius (see supplemental Table S1 for details).

Larvae of *H. armigera*, *M. brassicae*, *S. litura*, *H. tarsicrinalis*, *E. japonica* and *A. selenaria* were reared on a commercial artificial diet for silkworms, Silkmate™ 2M (Nosan Corp., Yokohama, Japan), and the other larvae were reared on their host plants under conditions of 24°C, 50–70% relative humidity, and a 16h:8h light:dark cycle. Pupae and/or adults were separated by sex, based on the genital morphology of the terminal abdominal segment, and maintained under the same environmental conditions as for rearing. Emerged female and male moths were transferred to 430 ml plastic cups with a supply of water until used.

Ultrasound recordings

A single unmated male and five to ten 1- to 4-day-old virgin females were introduced into a cubic mesh cage (18 cm × 18 cm × 18 cm) placed in a soundproof box (40 cm × 40 cm × 70 cm) with one side open, and maintained for >1 h before a recording. The activities of the insects were observed under a dim red light (0.2 lux). Sound recordings were made when the moths showed high mating activity, i.e. during the last 3 h of the scotophase. The exception was *C. suppressalis*, which showed high mating activity during the early scotophase (Samudra et al., 2002). Courtship sounds of male moths were recorded with a 1/4-inch (6.4 mm) condenser microphone [type 4939 (flat response from 3 kHz to 100 kHz with ±1 dB), Brüel & Kjær, Nærum, Denmark], the tip of which was placed 1 cm from the male. Signals from the microphone were amplified by pre- and conditioning-amplifiers [type 2670 and 2690 with a 0.02–100 kHz band-pass filter (–40 dB/decade), Brüel & Kjær]. The signals were digitized at a sampling rate of 300 kHz using a 14-bit A/D converter (Wavebook 512A, IOtech, Ohio, USA), and analyzed using the software BatSound 3.31 (Pettersson Elektronik AB, Uppsala, Sweden) after high pass filtering (>10 kHz, Butterworth filter). Power spectra of the sounds were computed using a Hanning window with an FFT (fast Fourier transformation) size of 1024 points. The peak equivalent sound pressure level (dB peSPL re. 20 µPa root mean square) of the sound was determined from the peak amplitude of the signal with reference to the signal of a sound level calibrator (type 4231, Brüel & Kjær; 94.00 ± 0.20 dB SPL at 1 kHz) (Stapells et al., 1982).

Comparison of sound pressure levels

To test our prediction that ultrasound signals used for courtship are less intense than those used in other contexts such as bat defense or mate attraction, sound pressure levels of moth ultrasounds reported to date were compared between two groups classified by the distance of communication, i.e. ‘close’ (within 5 cm) and ‘far’ (beyond 5 cm). Based on this criterion, species exhibiting close communication include *Galleria mellonella* (Spangler, 1985), *Plodia interpunctella* (Trematerra and Pavan, 1995) and *Ostrinia furnacalis* (Nakano et al., 2008), whereas species with far communication include *Rileyana* (*Thecophora*) *fovea* (Surlykke and Gogala, 1986),

Amyna natalis (Heller and Achmann, 1993), *Hecatesia exultans* (Alcock and Bailey, 1995), *Heliothis zea* (Kay, 1969), *Amphipyra perflua* (Lapshin and Vorontsov, 2000), *Syntomeida epilais* (Sanderford and Conner, 1995), *Cynia tenera* (Barber and Conner, 2006), *Arctia caja* (Surlykke and Miller, 1985), *Euchaetes egle* (Simmons and Conner, 1996), *Phragmatobia fuliginosa* (Surlykke and Miller, 1985), *Pyrrharctia isabella* (Krasnoff and Yager, 1988), *Euchaetes bolteri* (Simmons and Conner, 1996), *Empyreuma affinis* (Sanderford et al., 1998), *Bena bicolorana* (Skals and Surlykke, 1999), *Pseudoips prasinana* (Skals and Surlykke, 1999), *Corcyra cephalonica* (Spangler, 1987), *Achroia grisella* (Spangler, 1984), *Symmoracma minoralis* (Heller and Krahe, 1994), and 36 tiger moths described by Barber and Conner (Barber and Conner, 2006). Differences in the sound pressure levels emitted by the two groups were analyzed with a generalized linear model (GLM) using the software package R, version 2.7.0.

Behavioral experiments

To verify that the sounds were used for sexual communication in *S. litura*, we examined how mating was affected by deafening females. For the operation, 0- or 1-day-old virgin females were anesthetized with CO₂, and the tympanic membranes on both sides of the metathorax were punctured using a fine insect pin under a stereomicroscope. The sham operation involved puncturing another part of the metathorax. Behavioral experiments were conducted in the last 2 h of the scotophase 1 day after the operation. A single 1-day-old intact unmated male was introduced into a cubic mesh cage (25 cm × 25 cm × 25 cm), which housed 5–10 deafened, sham-operated, or intact females. Multiple females were housed in the cage so that always, at least one female would be releasing sex pheromone during the experiment. In response to the sex pheromone released by the females, the male usually readily flew up to one of the females, and started courting. The observation of mating behavior was continued until the female accepted the male or rejected him by flying away. The emission of ultrasounds by the male was continuously monitored with an ultrasound detector (model D240x, Pettersson Elektronik AB). The pair observed was removed from the cage, and the experiment was continued with a new male. The males that did not show courtship behavior within 5 min from the introduction were removed, and these were not included in the data.

RESULTS

In nine of 13 moth species examined, we detected the emission of ultrasounds by males at a specific step in a series of courtship behaviors. Here, it should be mentioned that sounds specifically emitted in association with courtship could be distinguished from those produced incidentally with wing beats or the movement of other body parts by the distinctly high energy level in the ultrasound range (>20 kHz) of the courtship sounds.

Ultrasonic production in Noctuidae

Males of *S. litura* and *H. tarsicrinalis* produced courtship ultrasounds (Fig. 1), whereas males of three other noctuid species, *H. armigera*, *M. brassicae* and *D. cremata*, did not produce sounds in the ultrasound frequency range (>20 kHz).

Males of *S. litura* produced a series of bursts of ultrasonic clicks during courtship (the lower oscillogram in Fig. 1A). The sound was broadband high frequency, ≈20–80 kHz, with a peak power frequency of 51 kHz and a sound level of 70 ± 2 dB peSPL at a distance of 1 cm (mean ± s.d., *N* = 15). The bursts lasted 9.5–16.0 ms and numbered ≈60 per second whereas the clicks lasted 0.1 ms and numbered ≈1900 per second. The number of clicks in one burst

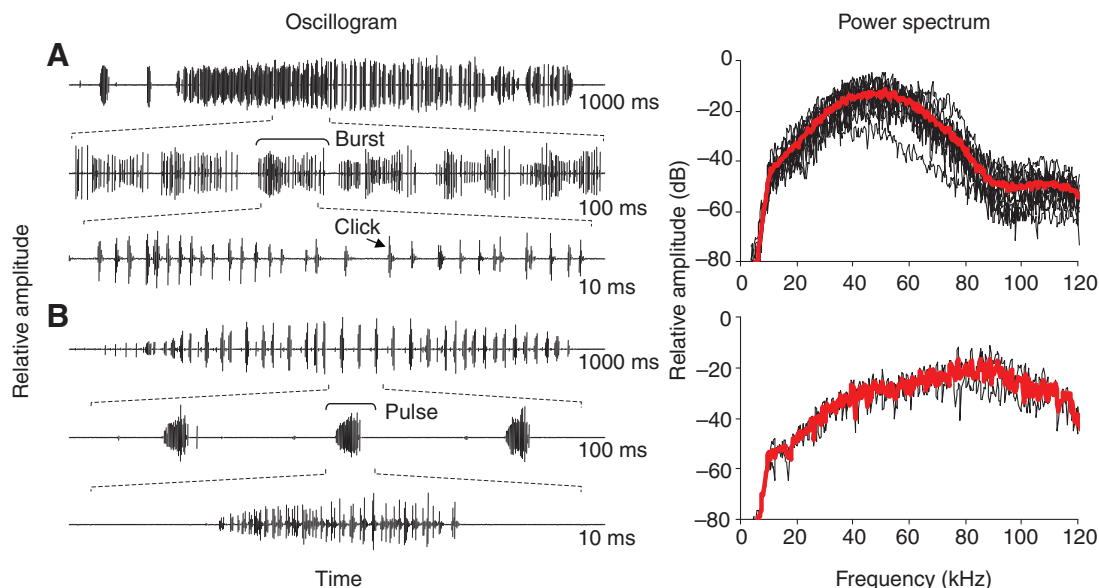


Fig. 1. Courtship ultrasounds of noctuid moths (Noctuoidea). Left: oscillograms of male courtship ultrasounds. Right: power spectra showing the frequency–amplitude distribution of the male sounds. Black lines are individual spectra and the red line, the average spectrum. (A) Ultrasounds of *Spodoptera litura* ($N=14$). A single song train, bursts of clicks and clicks are shown in the oscillograms. (B) Ultrasounds of *Herminia tarsicrinalis* ($N=2$). A single song train, three pulses and the structure of pulses are shown in the upper, middle and lower oscillograms, respectively.

was 36 ± 14 with fewer in the beginning of song trains (upper oscillogram of Fig. 1A).

In *H. tarsicrinalis*, males emitted trains of pulses with a duration of ≈ 6.5 ms (Fig. 1B shows a single train of pulses in the upper oscillogram, three pulses in the middle oscillogram, and a single pulse in the lower oscillogram). Single pulses were repeated at a frequency of around 26 pulses per second. Each pulse consisted of 50–70 irregular clicks. Power spectra of the clicks showed energy in a broad frequency range up to above 100 kHz with a peak power frequency of 88.8 kHz (64 ± 2 dB peSPL, $N=2$). The intensity of the clicks increased gradually from the beginning to end of the pulse.

Ultrasonic production in Arctiidae

Males of *S. punctarium* and *E. japonica* emitted ultrasounds during courtship (Fig. 2), whereas males of *H. cunea* did not.

Males of *S. punctarium* produced a regular pattern of pairs of ultrasonic clicks (Fig. 2A). A single click lasted 0.4–0.6 ms, and was repeated at 10–100 clicks per second. There was large individual variation in the sound level (66 ± 4 dB peSPL, $N=12$), but the first click of the pair (63 ± 5 dB peSPL, $N=12$) was always significantly weaker than the second (Wilcoxon signed rank test, $N=12$, $V=78$, $P=0.0025$). The peak power frequency of the clicks was around 38 kHz.

When close to a female, courting males of *E. japonica* emitted bursts of ultrasonic clicks with two to six, predominantly four, clicks

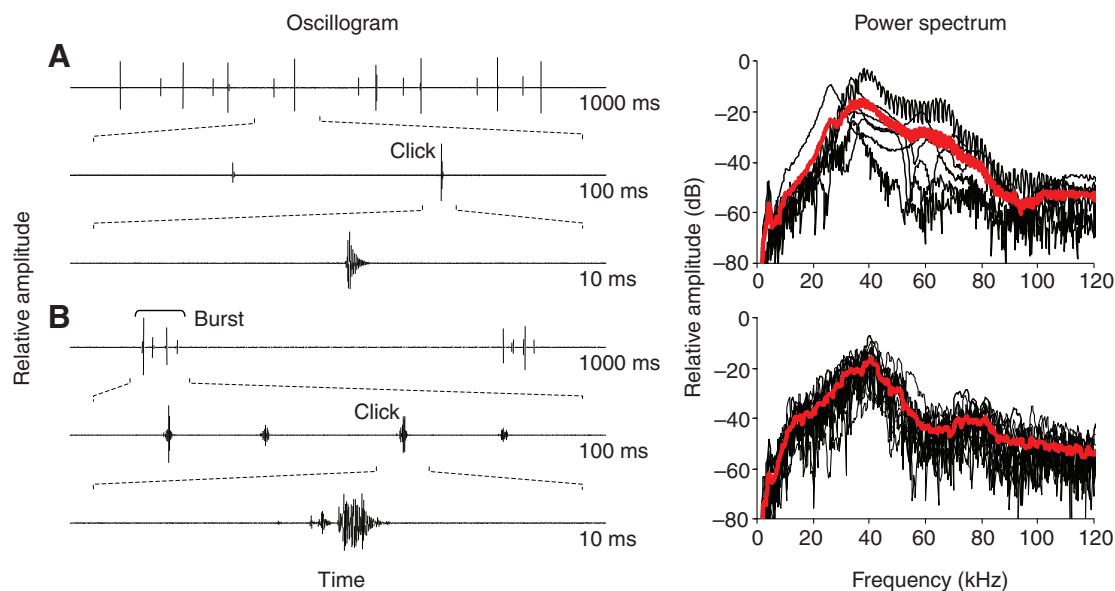


Fig. 2. Courtship ultrasounds of arctiid moths (Noctuoidea). Left: oscillograms of male courtship ultrasounds. Right: power spectra showing the frequency–amplitude distribution of the male sounds. Black lines are individual spectra and the red line, the average spectrum. (A) Ultrasounds of *Spilosoma punctarium* ($N=7$). A single pair of clicks is shown in the middle oscillogram. (B) Ultrasounds of *Eilema japonica* ($N=13$). Two bursts of clicks and single clicks are shown in the upper and lower oscillograms, respectively.

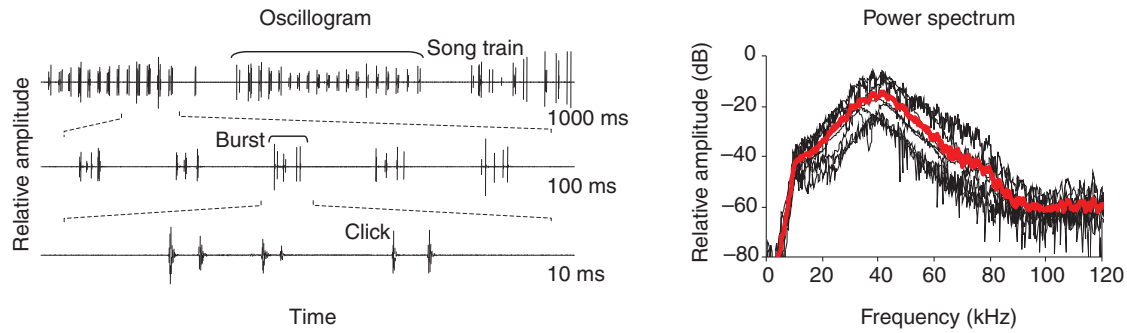


Fig. 3. Courtship ultrasound of a geometrid moth, *Ascotis selenaria cretacea* (Geometroidea) ($N=8$). Left: oscillograms of male courtship ultrasounds. Right: power spectra showing the frequency–amplitude distribution of the male sounds. Black lines are individual spectra and the red line, the average spectrum. Three song trains, five bursts of clicks and a single burst are shown in the respective oscillograms.

in a single burst (Fig. 2B). One burst lasted 50–85 ms, and was repeated *ca.* 1.2 times per second. The clicks had a peak power frequency of 41 kHz. Clicks lasted ≈ 2 ms with a repetition rate of 35–70 clicks per second ($N=13$). The first, third and fifth clicks of one burst were significantly more intense (62 ± 3 dB peSPL at 1 cm) than the second, fourth and sixth clicks (56 ± 3 dB peSPL; Wilcoxon signed rank test, $N=13$, $V=91$, $P=0.00024$).

Ultrasonic production in Geometridae

Males of *A. selenaria* produced bursts of clicks with a peak power frequency of 43 kHz and a maximum sound intensity of 73 ± 3 dB peSPL ($N=9$; Fig. 3). One song train lasted 200–600 ms and contained 10–30 bursts. One burst consisted of 4–6 clicks and lasted 6–10 ms.

Ultrasonic production in Crambidae

Males of all crambid moths tested, i.e. *C. suppressalis*, *G. pyloalis*, *P. nigropunctalis* and *S. recurvalis* emitted courtship ultrasounds (Fig. 4).

C. suppressalis males emitted ultrasounds with a peak power frequency of 52 kHz (76 ± 3 dB peSPL, $N=8$; Fig. 4A). A single train

(upper oscillogram of Fig. 4A) was composed of pulses (middle oscillogram of Fig. 4A) lasting ≈ 4 ms and repeated at 40–55 pulses per second. The number of clicks in single pulses ranged from 7 to 15.

Males of *G. pyloalis* produced an irregular series of ‘hyper’ ultrasonic clicks with peak energy above 100 kHz. Clicks were repeated on average at 1075 clicks per second. The duration was ≈ 0.13 ms and the peak power frequency was 113 kHz. The maximum sound level was $\approx 54 \pm 2$ dB peSPL at 1 cm ($N=6$; Fig. 4B).

P. nigropunctalis males emitted several bursts of clicks in one train (upper oscillogram of Fig. 4C). Single bursts lasting *ca.* 19 ms were repeated 16 times per second, and contained 10–14 clicks (middle oscillogram of Fig. 4C). The duration of single clicks within a burst was around 0.13 ms, the maximum intensity was $\approx 71 \pm 1$ dB peSPL, and the bandwidth was broad, ranging from 30 to 120 kHz with a peak around 100 kHz ($N=2$; Fig. 4C).

Males of *S. recurvalis* emitted discontinuous clicks lasting 0.12 ms at a high rate, 1140 clicks per second. The maximum sound level was $\approx 43 \pm 3$ dB peSPL, and the peak power frequency was around 115 kHz ($N=7$; Fig. 4D), hence with a bias toward hyper ultrasound over 100 kHz. Because frequencies over 100 kHz are

Table 1. Acoustic features of moth courtship songs

Family	Subfamily	Species*	Ear location	Sonic pattern†	Frequency (kHz)‡	Sound level (dB peSPL)§
Noctuidae	Hadeninae	<i>Spodoptera litura</i>	Metathorax	Click	51	69.9 ± 2.3
	Herminiinae	<i>Herminia tarsicrinalis</i>	Metathorax	Stridulation	89	63.9 ± 2.0
Arctiidae	Arctiinae	<i>Cynia tenera</i>	Metathorax	Click	50	106
		<i>Euchaetes egle</i>	Metathorax	Click	40	101
		<i>Euchaetes bolteri</i>	Metathorax	Click	30	93
		<i>Pyrrharctia isabella</i>	Metathorax	Click	≈ 36	96
		<i>Spilosoma punctarium</i>	Metathorax	Click	38	66.0 ± 4.0
	Lithosiinae	<i>Eilema japonica</i>	Metathorax	Click	41	61.6 ± 3.0
Geometridae	Ennominae	<i>Ascotis selenaria</i>	Abdomen	Click	43	72.7 ± 3.0
Pyrilidae	Galleriinae	<i>Galleria mellonella</i>	Abdomen	Click	72	80
	Phycitinae	<i>Plodia interpunctella</i>	Abdomen	Click	≈ 32	≈ 60
Crambidae	Crambinae	<i>Chilo suppressalis</i>	Abdomen	Stridulation	52	75.8 ± 3.3
	Pyraustinae	<i>Glyphodes pyloalis</i>	Abdomen	Click	113	54.2 ± 2.1
		<i>Ostrinia furnacalis</i>	Abdomen	Stridulation	45	46
		<i>Palpita nigropunctalis</i>	Abdomen	Click	102	70.5 ± 0.6
		<i>Spoladea recurvalis</i>	Abdomen	Click	115	43.1 ± 2.8

*Species shown in bold letters were examined in the present study.

†See text for the description of the patterns.

‡Peak power frequency of the sound.

§Sound level at a distance of 1 cm (mean \pm s.d.). The frequency of *P. isabella* and the frequency and sound level of *P. interpunctella* were estimated from previous publications (Krasnoff and Yager, 1988; Trematerra and Pavan, 1995). The sound levels of *G. pyloalis* and *S. recurvalis* are underestimated because of the specifications of the microphone over 100 kHz. Sample size: *S. litura*, 15; *H. tarsicrinalis*, 2; *S. punctarium*, 12; *E. japonica*, 13; *A. selenaria*, 9; *C. suppressalis*, 8; *G. pyloalis*, 6; *P. nigropunctalis*, 2; *S. recurvalis*, 7.

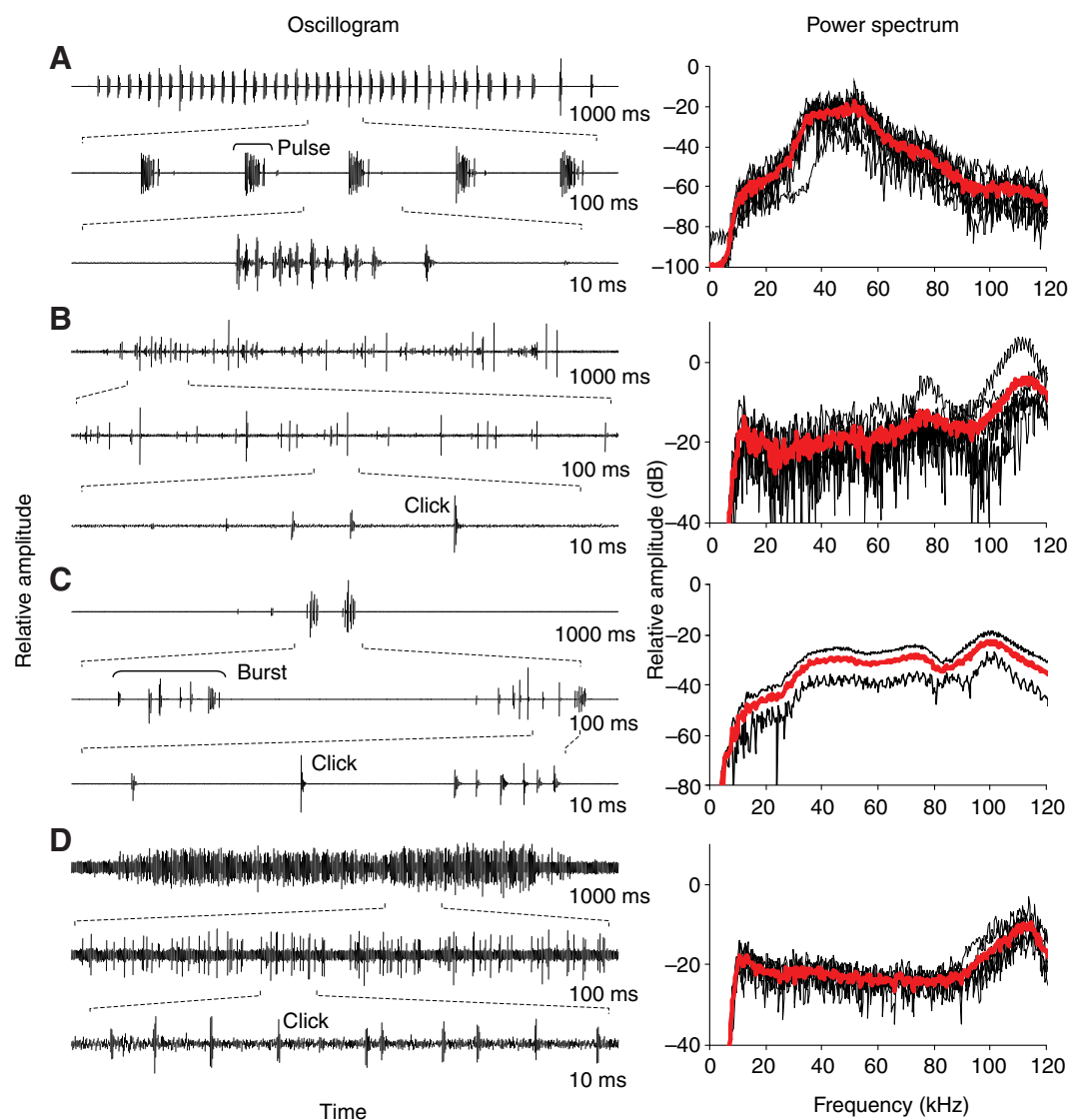


Fig. 4. Courtship ultrasounds of crambid moths (Pyralloidea). Left: oscillograms of male courtship ultrasounds. Right: power spectra showing the frequency–amplitude distribution of the male sounds. Black lines are individual spectra and the red line, the average spectrum. (A) *Chilo suppressalis* ($N=8$). A single song train, five pulses and a single pulse are shown in the respective oscillograms. (B) *Glyphodes pyloalis* ($N=6$). A single song train is shown in the upper oscillogram. (C) *Palpita nigropunctalis* ($N=2$). A single song train and two bursts of clicks are shown in the upper and middle oscillograms, respectively. (D) *Spoladea recurvalis* ($N=6$). A single song train is shown in the upper oscillogram.

beyond the specifications of the present recording system, maximum sound levels of *G. pyloalis*, *P. nigropunctalis* and *S. recurvalis* are likely to be underestimated.

Sound pressure levels of ultrasound in various moths

Reported sound levels of the acoustic signals produced by various moths differed widely in maximum energy from 43 to 125 dBpeSPL at 1 cm (Fig. 5A). The levels were not dependent on the mechanism of sound production or on the taxonomic group (family) to which the moth belongs [likelihood ratio (LR) test in generalized linear model (GLM), Gaussian error, identity link, mechanism: $\chi^2_{2,21}=41.5$, $P=0.87$; family: $\chi^2_{5,21}=981.9$, $P=0.30$]. The sound pressure levels of courtship ultrasounds were significantly lower than those of signals emitted for bat defense, mate attraction and territorial display (LR test in GLM, $\chi^2_{1,64}=7396.9$, $P<0.0001$; Fig. 5B).

Acoustic communication in *Spodoptera litura*

In *S. litura* (Noctuidae), males emitted ultrasound in association with courting. Deafening of females demonstrated the necessity for male ultrasound since males had substantially higher mating success with hearing than with deaf females; all of the intact females (100%,

$N=15$) and almost all the sham-operated females (93%, $N=15$) accepted the courting male, whereas only 52% of the deafened females ($N=21$) accepted the male.

DISCUSSION

To date, only a small number of moth species have been reported to produce ultrasounds for sexual communication (Conner, 1999; Greenfield, 2002). In the present study, we explored the possibility of weak ultrasound production by courting male moths. We showed that the production of weak ultrasounds is much more widespread among moths than previously assumed, because nine of 13 tested species produced sounds in close proximity to a female, after showing typical female sex pheromone-mediated mating behaviors (Yushima and Tamaki, 1974; Seol et al., 1986; Witjaksono et al., 1999; Samudra et al., 2002; Shirai, 2006).

The ultrasounds emitted by courting males varied extensively in temporal and spectral properties across species. The ultrasonic signals produced by males of *S. litura*, *S. punctarium*, *E. japonica*, *A. selenaria*, *G. pyloalis*, *P. nigropunctalis* and *S. recurvalis* consisted of single, often irregular clicks (Fig. 1A, Fig. 2, Fig. 3 and Fig. 4B–D), suggesting that these species have smooth tymbal organs as found in tiger and wax moths (Table 1) (Spangler, 1988; Fullard, 1992;

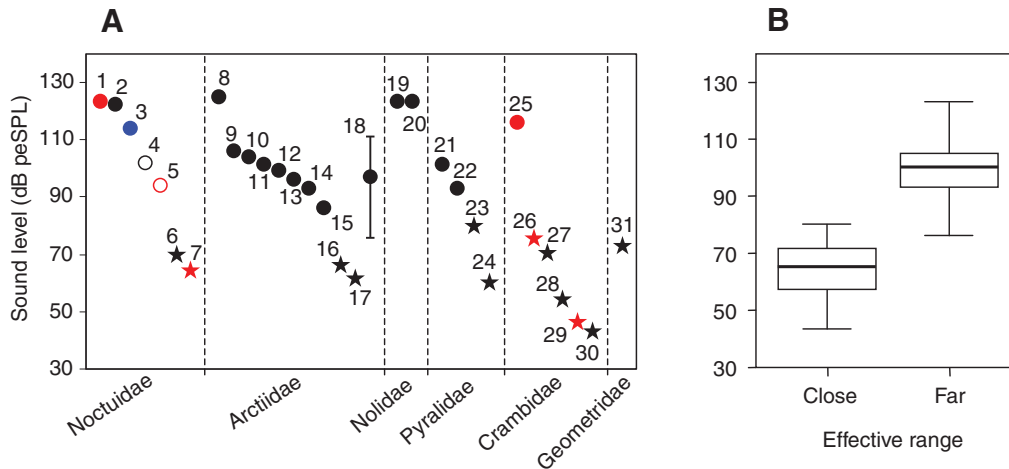


Fig. 5. Pressure levels of sounds emitted by various moths for mating, territorial display or bat-defense. (A) Scatter plot of the sound pressure levels (0 dB SPL = 20 μ Pa) at a distance of 1 cm from the moths, arranged by family. The levels (mean \pm s.d.) of each family are as follows: Noctuidae, 114 ± 24 dB peSPL, $N=7$ species; Arctiidae, 103 ± 11 dB peSPL, $N=46$ species; Nolidae, 123 ± 0 dB peSPL, $N=2$ species; Pyralidae, 92 ± 18 dB peSPL, $N=4$ species; Crambidae, 101 ± 27 dB peSPL, $N=6$ species; Geometridae, 73 dB peSPL, $N=1$ species. The color of symbols denotes the sound-producing mechanism: red, stridulation with a file-scraper; black, clicking with a tymbal; blue, percussion with an alar castanet [stridulation, 111 ± 30 dB peSPL (mean \pm s.d.), $N=6$ species; click, 105 ± 16 dB peSPL, $N=59$ species; percussion, 114 dB peSPL, $N=1$ species]. Filled circles: sound emissions for advertisement, territorial and bat-defense calls; open circles: unknown function at long distance; stars: sound emission for courtship calls at close distance. See B for descriptive statistics. Numbers near the symbols are the moth species: 1, *Rileyana (Thecophora) fovea*; 2, *Amyra natalis*; 3, *Hecatesia exultans*; 4, *Heliothis zea*; 5, *Amphipyra perflua*; 6, *Spodoptera litura*; 7, *Herminia tarsicrinalis*; 8, *Syntomeida epilais*; 9, *Cycnia tenera*; 10, *Arctia caja*; 11, *Euchaetes egle*; 12, *Phragmatobia fuliginosa*; 13, *Pyrrharctia isabella*; 14, *Euchaetes bolteri*; 15, *Empyreuma affinis*; 16, *Spilosoma punctarium*; 17, *Eilema japonica*; 18, 36 arctiid species studied by Barber and Conner (Barber and Conner, 2006) (filled circle and bar indicate median and range, respectively); 19, *Bena bicolorana*; 20, *Pseudopsis prasinana*; 21, *Corcyra cephalonica*; 22, *Achroia grisella*; 23, *Galleria mellonella*; 24, *Plodia interpunctella*; 25, *Symmoracma minoralis*; 26, *Chilo suppressalis*; 27, *Palpita nigropunctalis*; 28, *Glyphodes pyloalis*; 29, *Ostrinia furnacalis*; 30, *Spoladea recurvalis*; 31, *Ascotis selenaria*. Sound pressure levels, normalized at a distance of 1 cm, of species 1–17, 19–31 were obtained from Nakano et al. (Nakano et al., 2008; Nakano et al., 2009). (B) Difference in sound pressure levels of acoustic signals between effective ranges. 'Close' [64 ± 11 dB peSPL, $N=12$ species (64 ± 10 dB peSPL, $N=9$ species used in the present study), symbolized by stars in A] indicates sound emission within 5 cm during courtship, and 'far' (100 ± 11 dB peSPL, $N=54$ species, symbolized by circles in A) indicates emission of ultrasounds beyond 5 cm for advertisement to mates and rivals, and for avoidance of bat predation. Box-and-whisker plots show the median, lower and upper quartiles, and adjacent values within $1.5 \times$ interquartile ranges from the quartiles. A significant difference exists between the ranges (LR test in GLM, Gaussian error, identity link, $\chi^2_{1,64}=7396.9$, $P<0.0001$).

Conner, 1999). By contrast, the sounds produced by *H. tarsicrinalis* and *C. suppressalis* were not single clicks but regularly generated pulses (Fig. 1B and Fig. 4A) characterized by consecutive clicks, suggesting that the two species have stridulating organs such as the file-and-scraper found in *Rileyana fovea* (Noctuidae) (Surlykke and Gogala, 1986) and *Syntonarcha iriastis* (Crambidae) (Gwynne and Edwards, 1986) (Table 1). With respect to spectral properties, the frequency of maximum energy produced by males ranged broadly from 38 to 115 kHz (Table 1). All moths studied to date produce ultrasounds of less than 100 kHz except for *Achroia grisella* (Pyralidae) (Conner, 1999). It is worth noting that some crambid moths produced 'hyper' ultrasonic frequencies (over 100 kHz), a frequency range that only a few bats use for echolocation (Miller and Surlykke, 2001; Schnitzler and Kalko, 2001). Thus, the results indicate that moths belonging to different genera and families have evolved various mechanisms to emit ultrasounds during courtship (Table 1).

Our findings corroborate the prediction that many moths are not silent but emit quiet ultrasounds, probably keeping the sound level low to avoid eavesdropping by predators, parasites and rival males (Nakano et al., 2008; Nakano et al., 2009). Here we provided evidence for acoustic sexual communication in *S. litura*, since our results clearly showed that mate acceptance by females was much higher when she could hear the ultrasound produced by the male. We are continuing behavioral studies and have preliminary evidence that in some moth species examined in the present study, ultrasound does function in acoustic sexual communication.

We have suggested that a large proportion of moths communicate acoustically at close range using quiet ultrasound, which is audible to their mates but inaudible to unintended receivers. Moreover, the use of hyper-frequency ultrasonic signals is perhaps a good strategy to avoid detection by enemies because such high-frequency sounds are not utilized by most common predators of moths. This strongly suggests that acoustic communication using quiet ultrasound is much more widespread among hearing moths than hitherto presumed because it is so inconspicuous. We found that 70% of the moths we examined produced ultrasound, which suggests that in moths, close-range sound communication may be the norm rather than the exception, which has important consequences for our understanding of the co-evolution of bat and moth audition.

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Table S1. List of Japanese moth species used in this study

Superfamily	Family	Species	Collected stage and host plant	Site	Date
Noctuoidea	Noctuidae	<i>Helicoverpa armigera</i>	Larvae on <i>Cosmos bipinnatus</i> petals	Oumi-hachiman, Shiga (N35.1°, E136.1°)	Oct 2007
		<i>Mamestra brassicae</i>	Eggs on mulberry leaves	Bunkyo, Tokyo (N35.4°, E139.5°) (University of Tokyo)	Jul 2008
		<i>Spodoptera litura</i>	Larvae on <i>Ricinus communis</i> and mulberry leaves	University of Tokyo	Oct 2007 and 2008
		<i>Herminia tarsicrinalis</i>	Adult female*	Ushiku, Ibaraki (N35.6°, E140.1°)	May 2007
		<i>Diomea cremata</i>	Larvae and pupae on shiitake mushroom	Sodegaura, Chiba (N35.3°, E139.6°)	Sep 2008
	Arctiidae	<i>Spilosoma punctarium</i>	Larvae on mulberry leaves	Kamakura, Kanagawa (N35.2°, E139.3°)	Jul 2008
		<i>Hyphantria cunea</i>	Larvae on mulberry leaves	Moriya, Ibaraki (N35.6°, E139.6°)	Aug 2008
		<i>Eilema japonica</i>	Larvae on lichen and adult females*	University of Tokyo	Jun and Jul 2008
Geometroidea	Geometridae	<i>Ascotis selenaria</i>	Adult females*	University of Tokyo	May 2008
Pyraloidea	Crambidae	<i>Chilo suppressalis</i>	Larvae in rice stem	Moriya, Ibaraki	Aug 2008
		<i>Glyphodes pyloalis</i>	Larvae on mulberry leaves	University of Tokyo	Aug 2007
		<i>Palpita nigropunctalis</i>	Larvae on <i>Ligustrum japonicum</i> leaves	University of Tokyo	Apr 2008
		<i>Spoladea recurvalis</i>	Larvae on <i>Echinochloa crus-galli</i> and <i>Achyranthes bidentata</i> leaves	University of Tokyo	Aug 2007

*Mated females were captured with a sweep net, and their offspring were used in the experiments.